Here we are employing a new approach called the String Method with swarms-of-trajectories to study transition pathways for various zwitterionic and anionic molecules across both WT and mutant OmpF porins. Results of this work will therefore assist in the design of new antibiotics that are more effective in the treatment of bacterial infections. [Supported by NIH grant GM062342].

3564-Pos

Towards a Cell-Free Assay to Investigate Lipid Bilayer Permeation and Efflux Transport of Therapeutic Agents

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Lipid bilayers and efflux transporters like P-glycoprotein (P-gp) represent the most important *in vivo* barriers for therapeutic agents. Driven by ATP hydrolysis, P-gp exports structurally diverse hydrophobic compounds from the cell reducing intestinal absorption and blood-brain barrier passage. P-gp expression has also been linked to the efflux of chemotherapeutic drugs in human cancer cells, contributing to multidrug resistance.

The aim of this project is to adapt a liposomal permeation assay to study lipid bilayer permeation and P-gp transport in parallel. This is achieved by integrating P-gp into liposomes.

Fully functional His-tagged P-gp was overexpressed in HEK293 cells and purified by immobilized metal affinity chromatography. P-gp was reconstituted into liposomes, incorporation was verified by density gradient centrifugation. 90% of the basal ATPase activity originated from P-gp as determined with an anti-Pgp antibody. Cryo-TEM images showed unilamellar vesicles with a homogenous size distribution.

In parallel, we established liposomal assays to investigate membrane partitioning and permeation with the pH-sensitive probe fluorescein, linked to the phospholipid DHPE. A minute pH-shift at the membrane surface due to weak acids or bases entering or crossing the lipid bilayer results in a characteristic change in fluorescence. Well-known P-gp substrates and non-substrates were studied. The resulting fluorescence time-curves followed mono- or biexponential functions and the rate constants of the faster terms were used to calculate the apparent permeation coefficients ($Perm_{app}$). The $Perm_{app}$ values of the compounds investigated do not correlate with their lipophilicity or other physico-chemical parameters, which are generally used to estimate membrane permeation. The data obtained strongly emphasizes the importance of developing a simplified permeation assay.

We thank the OPO Foundation Switzerland and the Electron Microscopy Center Zurich.

3565-Pos

Classification of Human Solute Carrier Superfamily Members Reveals Functional Similarities across Families

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Solute Carrier (SLC) superfamily members are membrane transporter proteins that control the uptake and efflux of solutes, including essential cellular compounds, environmental toxins and therapeutic drugs, across biological membranes. Members of the SLC superfamily can share surprisingly similar structural features despite weak sequence similarities. Identification of sequence relationships among SLC members is needed to enhance our ability to model individual transporters and to elucidate the molecular mechanisms of their substrate specificity and transport.

Here, we describe a comprehensive sequence-based classification of SLC members into families. We classify the proteins using sensitive profile-profile alignments and two classification approaches, including similarity networks. The clusters are analyzed in view of substrate specificity, transport mode, organism conservation, and tissue specificity. SLC families with similar substrates generally cluster together, despite exhibiting relatively weak sequence similarities. In contrast, some families cluster together with no apparent reason, revealing unexplored relationships. We demonstrate computationally and experimentally the functional overlap between representative members of these families. Finally, we identify 4 putative SLC transporters in the human genome.

The SLC superfamily constitutes a biomedically important family of membrane proteins that is highly diverse in sequence. The proposed classification of the superfamily, combined with experiment, reveals new relationships among the individual families and identifies new superfamily members. The classification scheme will inform future attempts directed at modeling the structures of the SLC transporters, a prerequisite for describing their substrate specificity.

3566-Pos

Model-Structure, Mutagenesis and Functional Characteristics of the Human Transporter, NHA2

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Human NHA2 is a novel member of the Cation/Proton Antiporters-2 (CPA2) family, linked to essential hypertension. Using the crystal structure of distant bacterial transporter NhaA as template, producing a model-structure of NHA2 necessitated a composite modeling approach. Through extensive mutagenesis guided by our model, we show that while NHA2 retained some functional and structural core elements of other Na+/H+ exchangers, it exhibited other significant exclusive features. A cluster of highly conserved titratable residues was located in the so-called assembly region, made of two discontinuous helices TM4 and TM11. Whereas in NhaA, oppositely charged residues have been proposed to compensate for partial dipoles generated within this assembly, we demonstrate that in NHA2 uncharged but polar residues suffice. Instead, NHA2 possesses unique, conserved charges predicted to interact with key essential residues. Combining structural data with evolutionary conservation analysis and mutagenesis, we propose a transport mechanism for NHA2, and compare it with mechanisms proposed for NhaA and NHE1. This study illustrates an attractive approach for studying new transporters, starting from structural data to guide initial experimental efforts.

3567-Pos

Functional Reconstitution of a Bicomponent ABC Transporter

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The ATP-binding-cassette (ABC) transporters are transmembrane protein nanomachineries present in all living systems. ABC transporters utilize the energy of ATP hydrolysis to transport a variety of solutes across the membrane. *Pseudomonas aeruginosa*, a Gram-negative pathogenic bacterium, employs a bi-component ABC transporter as an active efflux of polysaccharides during the biogenesis of endotoxic lipopolysaccharides. We reconstituted the ABC transporter in various systems, including microsomes, planar lipid bilayers, and transfected mammalian N2a cell lines to obtain a mechanistic understanding of the functional properties of this nanomachinery. We employed single-channel electrical recordings to show that the transmembrane domain (TMD) of the ABC transporter features pore-forming activity. Further, our biochemical characterization of purified components sheds light on the structural assembly and stoichiometry of this bi-component ABC transporter. Our long-term goal is to detect, explore and characterize the translocation of polysaccharides at single-protein complex resolution. The use of a broad range of reconstitution systems enables a comprehensive examination of the ATP-dependent transport kinetics and thermodynamics of the large-size substrates from one side of the membrane to the other. These studies might also contribute to drug design against *Pseudomonas aeruginosa.*

Acknowledgements. This research was supported by grants from the National Science Foundation (DMR-0706517 and HRD-0703452) and the National Institutes of Health (R01 GM088403) as well as by Syracuse Biomaterials Institute (SBI) for Liviu Movileanu and the National Institutes of Health (HL042220) for Richard Veenstra.

3568-Pos

New Immobilized Proteoliposome-Based Biosensor System for Investigating Human ATP-Binding Cassette Transporters

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ATP-binding cassette (ABC) transporters comprise a large family of membrane proteins that transport a variety of organic substrates across cellular membranes. Human ABC transporters are mostly efflux pumps for physiological and xenobiotic substrates related to immune response and cellular detoxification. Nine human ABC transporters play a major role in cellular multi-drug resistance (MDR), thus being called MDR-related proteins (MRPs). So far, the structure of MRPs and human ABC transporters in general is unclear. Sequence analysis and experimental data indicate that functional ABC transporters are composed of two subunits and imply strong positive cooperativity between those entities. To elucidate the transport mechanism and the molecular origin of the cooperativity a solid-supported